



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/GB99/02446 <b>(22) International Filing Date:</b> 27 July 1999 (27.07.99)  <b>(30) Priority Data:</b> 9816358.7                   27 July 1998 (27.07.98)                   GB 60/096,528               13 August 1998 (13.08.98)                   US  <b>(71) Applicant (for all designated States except US):</b> ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P ANGELETTI S.P.A. [IT/IT]; Via Pontina Km 30.600, I-00040 Pomezia (IT).  <b>(71) Applicant (for MN only):</b> NICHOLLS, Kathryn, Margaret [GB/GB]; 32 Somerset Street, Bristol BS2 8LY (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> ALTAMURA, Sergio [IT/IT]; Viale Tito Livio, 95, I-00136 Rome (IT). TOMEI, Licia [IT/IT]; Via C.E. Gadda, 173, I-00143 Rome (IT). KOCH, Uwe [DE/IT]; Via Orti Pompei, 23, I-00041 Albano Laziale (IT). NEUNER, Philippe, Jean, Siegfried [FR/IT]; Via Pratolungo, 11B, I-00041 Albano Laziale (IT). SUMMA, Vincenzo [IT/IT]; Via Panoramica, 36, I-00049 Velletri (IT).		<b>(74) Agents:</b> NICHOLLS, Kathryn, M. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).  <b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> DIKETOACID-DERIVATIVES AS INHIBITORS OF POLYMERASES  <div style="text-align: center;"> <math display="block">\text{R}-\text{C}(=\text{O})-\text{CH}_2-\text{C}(=\text{O})-\text{CO}_2\text{H}</math> <b>(A)</b> </div> <b>(57) Abstract</b> <p>Diketoacids of Formula (A) are useful as inhibitors of viral polymerases. In particular hepatitis C virus RNA dependent RNA polymerase (HCV RdRp), hepatitis B virus polymerase (HBV pol) and reverse transcriptase of human immunodeficiency virus (HIV RT). The group R may be broadly chosen and is an organic moiety which contains 2 to 24 carbon atoms and includes an optionally cyclic or heterocyclic group in which the atom directly bonded to the adjacent carbonyl in the diketoacid is part of the ring structure.</p>		

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## DIKETOACID-DERIVATIVES AS INHIBITORS OF POLYMERASES

Technical Field

The present invention relates to compounds useful as enzyme inhibitors, in particular as inhibitors of enzymes involved in the transfer of phosphoryl groups and, especially as inhibitors of polymerases. The invention further relates to pharmaceutical compositions containing such compounds, and to their use in the treatment of viral infections.

15

Polymerases are the enzymes which catalyse the formation of phosphodiester bonds in RNA and DNA. They play an essential role in viral replication and, therefore, are an important target in the fight against viral diseases such as human immunodeficiency virus (HIV), hepatitis, and poliomyelitis.

20

Background Art

US 5 475 109 describes dioxobutanoic acids substituted with piperidine or similar N-substituted saturated cycloalkyls as inhibitors of the cap-dependent endonuclease of influenza virus.

25

Disclosure of the Invention

The present inventors have discovered that a range of

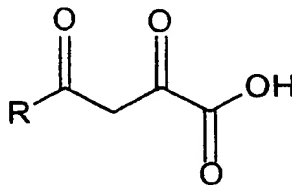
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5 diketoacids have utility as enzyme inhibitors and, in particular, as polymerase inhibitors and more particularly as inhibitors of hepatitis C NS5 RNA-dependent RNA polymerase, HBV DNA-dependent RNA polymerase and HIV DNA-dependent DNA polymerase. Their  
10 investigations indicate that these compounds may act by interfering with the binding of phosphoryl groups at the active site of the enzyme and may, therefore, have broad application in inhibiting enzymes involved in the transfer of phosphoryl groups.

15

According to a first aspect of the present invention there is provided a compound of formula A shown below. This compound is suitable for therapeutic use, for instance as an enzyme inhibitor.

20



## FORMULA A

Optionally, the compound may be in the form of a pharmaceutically acceptable salt or ester, which can be  
25 hydrolysed in vivo to the corresponding diketoacid.

5 In formula A, the group R is an organic moiety which  
contains from 2 to 24, preferably 4 to 20, most  
preferably 6 to 17 carbon atoms in total. R includes an  
optionally substituted cyclic or heterocyclic group in  
which the atom directly bonded to the adjacent carbonyl  
10 in the diketoacid is part of the ring structure.  
Preferably, this atom is a carbon atom.

The ring which is thus bonded to the carbonyl group is  
preferably a 3 to 8 membered ring, particularly a 4 to 6  
15 membered ring.

Thus, for example, R may be selected from:

- 20 (i) optionally substituted aromatic groups,  
especially those including six membered rings,  
such as phenyl and naphthyl;
- (ii) optionally substituted heteroaryl groups  
especially those including five and six  
25 membered rings such as thiophene, pyrrole,  
furan, imidazole, pyridyl, pyrimidyl, and  
pyridazyl; the heteroaryl ring may, optionally  
be fused to another ring;
- 30 (iii) optionally substituted cycloalkyl groups,

5 especially those including five or six membered  
rings such as cyclopentyl, cyclohexyl and  
adamantyl;

(iv) optionally substituted cycloalkenyl groups,  
10 especially those including five or six numbered  
rings such as cyclohexenyl, cyclopentenyl;

(v) optionally substituted cyclic heteroalkyl  
groups, especially those including five or six  
15 numbered rings such as piperidyl, pyrrolidyl,  
tetrahydrofuranyl, and tetrahydropyranyl; in  
this class 4- piperidyl rings substituted with  
an aryl group at carbon 4 and on acyl or  
sulfonyl substituent at N1 are preferred.

20

In the case of optional substitution, one or more  
substituents may be present and a wide variety of  
substituents are possible. Preferred optional  
substituents for all compounds of the present invention  
25 are set out in the following list:

- (a) -OH;
- (b) -SH;
- (c) - halogen, such as fluorine, chlorine or bromine,
- 30 (d) - CO<sub>2</sub> H;

- 5 (e) - CN;
- (f) - NO<sub>2</sub> ;
- (g) - NR<sub>1</sub>R<sub>2</sub> wherein each of R<sub>1</sub> and R<sub>2</sub> is selected from H and lower alkyl groups having 1 to 6 carbon atoms; or R<sub>1</sub> and R<sub>2</sub> together form a ring including 4 to 6 carbon atoms;
- 10 (h) - SO<sub>2</sub> NR<sub>1</sub>R<sub>2</sub> where R<sub>1</sub> and R<sub>2</sub> are as defined above;
- (i) -CONH<sub>2</sub>, -NHCO<sub>2</sub>H, or -NHCOCOOH;
- (j) an alkyl (or alkenyl or alkynyl group) group having 1 to 12 (2 to 12) carbon atoms, preferably 1 to 7 (2 to 7) carbon atoms optionally substituted by any one or more of the groups (a) - (i) above and/or optionally interrupted by a group selected from -O-, -S-, -NR<sub>3</sub> -,
- 15
- 20  $\begin{array}{c} \text{O} \\ \parallel \\ \text{-C-} \end{array}$  , -CO<sub>2</sub> -, -OCO-, -CONR<sub>3</sub> -, -NR<sub>3</sub>CONR<sub>3</sub>-, -SO<sub>2</sub> -, -NR<sub>3</sub>SO<sub>2</sub>-, and -SO<sub>2</sub> NR<sub>3</sub> -; where each R<sub>3</sub> independently is H or lower alkyl of 1 to 6 carbon atoms;
- (k) an aryl or heteroaryl group having 2 to 10 carbon atoms optionally substituted with any one or more of groups (a) to (j) above;
- 25
- (l) an aralkyl or heteroaralkyl group having 3 to 16 carbon atoms optionally substituted with any one or more of groups (a) - (j) above and/or in which the
- 30

5 alkyl part of the group is optionally interrupted by  
a group selected from

10  $-O-$ ,  $-S-$ ,  $-NR_3-$ ,  $-C(=O)-CO_2-$ ,  $-OCO-$ ,  $-CONR_3-$ ,  $-NR_3CONR_3-$ ,  $-SO_2-$ ,  $-NR_3SO_2-$ , and  $-SO_2NR_3-$ ; where  
 $R_3$  is as defined above;

15 (m)  $-C(=O)-R_4$  where  $R_4$  is an alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, or heteroaralkyl group as such groups are defined above at (j), (k) and (l);

20 (n)  $-C(=O)-O-R_4$  or  $-O-C(=O)-R_4$  where  $R_4$  is as defined above;

(o)  $-OR_4$  where  $R_4$  is as defined above;

25 (p)  $-C(=O)NHR_4$ ,  $-NH-C(=O)-R_4$  or  $-NH-C(=O)NHR_4$  where  $R_4$  is as defined above;

(q)  $-SO_2R_4$  where  $R_4$  is as defined above;

(r)  $-NHR_4$  or  $-N(R_4)_2$  where  $R_4$  is as defined above;

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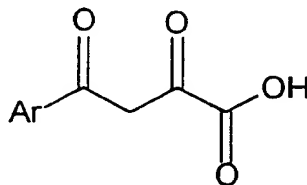
5 (s)  $-\text{NHSO}_2\text{R}_4$  or  $-\text{SO}_2\text{NHR}_4$ , where  $\text{R}_4$  is as defined above;

(t)  $-\text{SR}_4$

and each of optional substituents (j) to (t) above may  
10 optionally itself be substituted by one or more groups  
selected from (j) to (t).

A preferred class of compounds of formula A is  
represented by formula E:

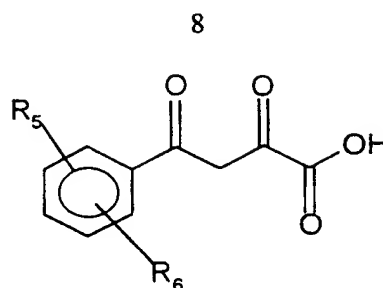
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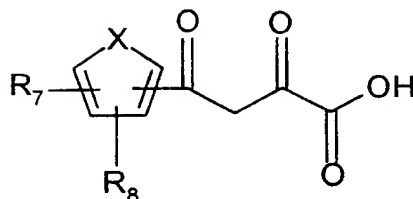
FORMULA E

in which Ar is an optionally substituted aryl or  
heteroaryl group. Optional substituents may be selected  
20 from the list of preferred substituents set out above.  
Within this class of preferred compounds two especially  
preferred groups are set out below (formulas F and G)

5



FORMULA F



10

FORMULA G

$R_5$ ,  $R_6$ ,  $R_7$  and  $R_8$  are, independently H or are selected from the optional substituents listed above and  $R_7$  and  $R_8$  taken together may form a 4 to 7, preferably 5 or 6 membered ring; and X is O, S, NH, or  $NR_4$  where  $R_4$  is as defined above.

15

In compounds of formula F, (which are optionally substituted phenyl diketoacids) ortho, meta and para

5 substitution are possible.

In general, it is preferred that there is a single substituent, preferably at the position which is ortho- or meta- to the diketoacid group. Substitution at the meta-position is especially preferred. Where two substituents are present, then preferably the phenyldiketoacid is 2,5-substituted; 3,5-substitution is also possible, as is 2,4-substitution provided, in the latter case, that the substituent at the 4-position is relatively small (e.g. methyl). Disubstitution at the 2,3- and 2,6-positions is, in general, not preferred.

Preferred substituents, especially at the ortho and meta positions, are ether groups of formula (o) above (i.e. -OR<sub>4</sub>), hydroxyl, and -NHSO<sub>2</sub>R<sub>4</sub>. It is generally preferred that no more than one substituent be -OR<sub>4</sub> and/or -NHSO<sub>2</sub>R<sub>4</sub>.

Preferred examples of -OR<sub>4</sub> groups which may be found at the ortho and meta positions and particularly at the meta position include:

-OCH<sub>2</sub>Ar or, less preferably -O(CH<sub>2</sub>)<sub>2</sub>Ar where Ar is an optionally substituted aryl or heteroaryl group and is particularly preferably an optionally substituted phenyl

5 group. Examples of preferred substituents on the aryl group, and especially on the phenyl ring include halogens, especially fluorine and chlorine, and electron-withdrawing groups such as -CN, -CO<sub>2</sub>H, and -CF<sub>3</sub> as well as ether and aryl groups;

10 -O-(CH<sub>2</sub>)<sub>3</sub>-CN; and  
-O-(CH<sub>2</sub>)<sub>3</sub>-C≡CH.

Preferred sulfonamide groups which may be found at the ortho- and meta- positions, particularly at the meta-  
15 position are those of formula:

-NH-SO<sub>2</sub>-Ar, where Ar is an optionally substituted aryl or heteroaryl group, preferably an optionally substituted phenyl group. Preferred optional substituents for the  
20 aryl, preferably phenyl group, include: -CN; halogens, especially chlorine and fluorine, -CF<sub>3</sub>, lower (C<sub>1-6</sub>) alkyl (especially methyl), hydroxy-, ether, and -NO<sub>2</sub> groups.

For both the -OCH<sub>2</sub>Ar and -NHSO<sub>2</sub>Ar substituted compounds,  
25 another preferred example of Ar is naphthyl.

Other preferred substituents at the ortho and meta positions are lower (eg C<sub>1-6</sub>) alkyl groups, especially C<sub>1-4</sub> alkyl, such as methyl and ethyl, but in particular  
30 methyl, aralkyl groups, especially phenylmethyl groups,

5 optionally substituted in the phenyl ring, especially by  
a halogen, and nitrogen-containing substituents such as  
primary, secondary or tertiary amine groups, optionally  
in protonated form, amide, urethane, or urea groups in  
each of which examples there is a nitrogen atom bonded to  
10 the phenyl ring.

One particularly preferred sub class of compounds of  
formula F is those in which each of  $R_5$  and  $R_6$  is  
selected from H, HO-,  $R_4$  O-, and  $-NHSO_2R_4$  provided that  
15 no more than one of  $R_5$  and  $R_6$  is  $R_4$  O- or  $-NHSO_2R_4$ .

In compounds of formula G the diketoacid group may be at  
the 2- or 3- position of the ring. In many cases  
20 substitution at the 2-position is preferred.

Preferred examples of compounds of formula G are those in  
which the five membered aromatic ring,



25 is a pyrrole or thiophene ring. In the case of the  
pyrrole-substituted diketoacids, the groups  $R_7$  and  $R_8$  may  
both be hydrogen and in many cases that is preferred. If  
 $R_7$  and  $R_8$  correspond to substituent groups, then these may

5 be at any of the positions not already occupied by the diketoacid group. Examples of possible substituents include alkyl (especially methyl), halogen, and aralkyl (especially benzyl) groups.

10 One embodiment of pyrrole substituted diketoacid is that in which the diketoacid group is at the 2- position of the ring and where the only other substituent in the ring is on the nitrogen atom. In this case, preferred examples of the substituent  $R_4$  present on the nitrogen  
15 atom, include alkyl, aryl or aralkyl groups, particularly aralkyl (such as benzyl) groups. Where an aryl or aralkyl group is present these are preferably substituted with halogen atoms, such as fluorine or chlorine, or by cyano-groups.

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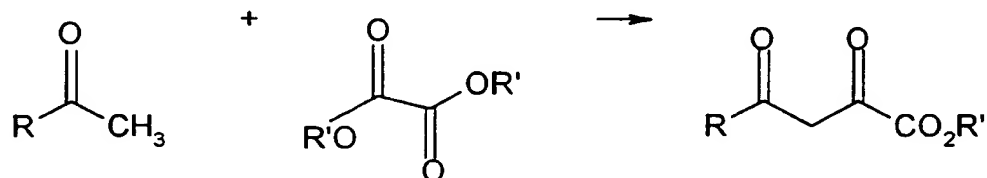
In the case of the thiophene-substituted diketoacids a wide range of substituents  $R_7$  and  $R_8$  may be employed in various positions as will be evident from the tables  
infra. Preferred thiophenes have an aralkyl (such as  
25 optionally substituted benzyl) or aryl (such as optionally substituted phenyl) substituent, e.g. at the 5-position of the thiophene ring.

Compounds containing furanyl rings may also be useful,  
30 especially for inhibiting HIV reverse transcriptase.

5 Preferred substituents are optionally substituted aryl groups (especially optionally substituted phenyl). Substitution is preferably at the 5-position of the ring.

The formulae of numerous preferred specific compounds of  
10 the present invention are presented later below.

The compounds of the present invention having formula A may be prepared by a process which comprises reaction of a compound of formula B with a dialkyloxalate of formula  
15 C followed by hydrolysis of the resulting diketo-ester of formula D:



FORMULA B

FORMULA C

FORMULA D

20 where R' is an alkyl group, typically having 1-6 carbon atoms. In the case where the target molecule is a pharmaceutically acceptable ester of the compound of formula A then R' in formula C may be selected accordingly, and the step of hydrolysing the compound of  
25 formula D omitted, since in vivo hydrolysis can render

5 the compounds active.

Preferred enzymes for inhibition by the compounds of the invention are those involved in phosphate transfer, in particular polymerases such as DNA polymerases, and RNA  
10 polymerases both of which may be either RNA dependent or DNA dependant. Compounds of the invention may particularly preferably be employed in the inhibition of viral enzymes. Examples of viral enzymes include RNA - dependent RNA polymerase and reverse transcriptases.

15

The compounds of the invention may be used as inhibitors of plant or animal (including human) viruses.

The viruses may be RNA viruses, which may, for example,  
20 be positive single stranded viruses of which polio virus, hepatitis C virus and encephalomyocarditis are examples, negative single stranded viruses such as orthomyxoviruses, and paramyxoviruses, and retroviruses of which HIV is a prominent example. Alternatively, the  
25 viruses may be DNA viruses, especially double stranded DNA viruses such as hepatitis B virus. In particular, compounds of the present invention may inhibit one or more of the following enzymes: hepatitis C virus RNA dependent RNA polymerase (HCV RdRp), hepatitis B virus  
30 polymerase (HBV pol) and reverse transcriptase of human



5 immunodeficiency virus (HIV RT).

Especially preferred compounds of the invention will be suitable for use as HCV RdRp inhibitors.

10 Other classes of enzyme involved in phosphate transfer which may be susceptible to inhibition by compounds of the present invention include phosphatases, Rnases, integrases and ribozymes.

15 According to a further aspect of the invention there is provided the non-therapeutic use of compound of formula A or suitable salt or ester as an enzyme inhibitor, especially as an inhibitor of polymerases, especially viral polymerases. For instance, compounds of the  
20 invention may be of utility in agriculture and horticulture for treating plants infected with or susceptible to plant virus.

According to a further aspect of the invention there is  
25 provided the use of a compound of formula A or of a pharmaceutically acceptable salt or ester thereof in the manufacture of a medicament for treatment of a viral illness in a human or animal. For instance, the medicament may be used to treat viral illness by  
30 inhibiting one or more viral polymerase. Preferably the

5 medicament is for treatment of hepatitis, such as hepatitis B or C, particularly hepatitis C, and human immunodeficiency virus.

A still further aspect of the invention provides a  
10 pharmaceutical composition comprising a compound of formula A, or a pharmaceutically acceptable salt or ester thereof and a pharmaceutically acceptable excipient, diluent or carrier. The composition may be in any suitable form, depending on the intended method of  
15 administration. It may for example be in the form of a tablet, capsule or liquid for oral administration, or of a solution or suspension for administration parenterally.

The pharmaceutical compositions optionally also include  
20 one or more other agents for the treatment of viral infections such as an antiviral agent, or an immunomodulatory agent such as  $\alpha$ -,  $\beta$ -, or  $\gamma$ - interferon.

A still further aspect of the invention provides a method  
25 of inhibiting an enzyme, especially a viral polymerase and/or of treating or preventing a viral illness, the method involving administering to a human or animal (preferably mammalian) subject suffering from the condition a therapeutically or prophylactically effective  
30 amount of the pharmaceutical composition described above

5 or of a compound of formula A or salt or ester thereof.  
"Effective amount" means an amount sufficient to cause a  
benefit to the subject or at least to cause a change in  
the subject's condition.

10 The dosage rate at which the compound, salt or ester is  
administered will depend on the nature of the subject,  
the nature and severity of the condition, the  
administration method used, etc. Appropriate values are  
selectable by routine testing. The compound, salt or  
15 ester may be administered alone or in combination with  
other treatments, either simultaneously or sequentially.  
For instance, it may be administered in combination with  
effective amounts of antiviral agents, immunomodulators,  
anti-infectives, or vaccines known to those of ordinary  
20 skill in the art. It may be administered by any suitable  
route, including orally, intravenously, cutaneously,  
subcutaneously, etc. It may be administered directly to  
a suitable site or in a manner in which it targets a  
particular site, such as a certain type of cell.  
25 Suitable targeting methods are already known.

A further aspect of the invention provides a method of  
preparation of a pharmaceutical composition, involving  
admixing one or more compound of formula A or salt or  
30 ester thereof with one or more pharmaceutically

- 5 acceptable adjuvants, diluents or carriers and/or with one or more other therapeutically or prophylactically active agents.

Modes for Carrying Out the Invention

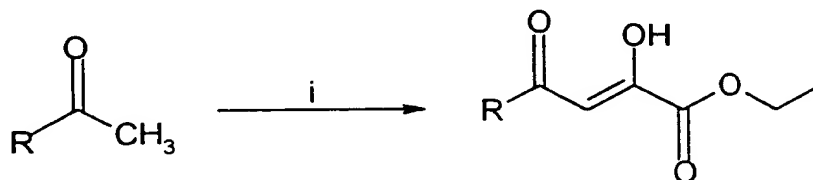
- 10 Embodiments of the invention are described below by the way of example only.

EXAMPLES

- 15 (1) Synthesis

The synthesis of the 2,4-dioxobutanoic acids consists of a Claisen condensation reaction between a methyl ketone substrate and diethyl oxalate in the presence of sodium ethoxide in tetrahydrofuran (Scheme 1A) and the subsequent hydrolysis of the ethyl ester with sodium hydroxide in methanol (Scheme 1B)

Scheme 1A

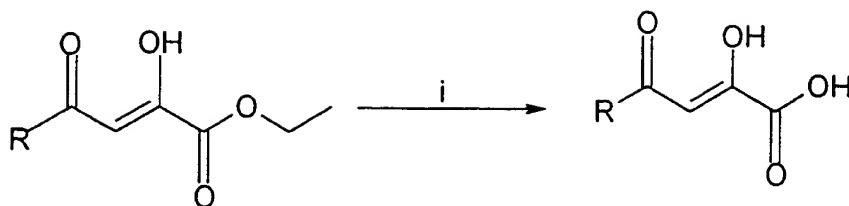


Reagents: (i) diethyl oxalate/NaOEt in THF

- 25 Scheme 1B

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Reagents: (i) 5eq. NaOH/MeOH

Exemplary procedure for the synthesis of the 2,4-dioxobutanoate ethyl esters

10 (Scheme 1A)

In a 50 ml round bottom flask with a stirring bar and under an inert atmosphere, the methyl ketone compound (1.0 mmole) in 10 ml of dry tetrahydrofuran (THF) is reacted with 2 equivalents of diethyl oxalate and 2 equivalents of sodium ethoxide (NaOEt) at ambient temperature for 3 hours. When reaction is completed, the reaction mixture is poured into a 1N aqueous hydrochloric acid (HCl) and extracted with ethyl acetate (EtOAc). The organic phase is separated, washed first with water and then with brine. The organic layer is dried over sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), filtered and solvent is removed in vacuo leaving the desired dioxobutanoate ethyl ester in quantitative yield.

### Exemplary procedure for hydrolysis of the ethyl ester

5 (Scheme 1B)

In a 50 ml round bottom flask with a stirring bar, the  
2,4-dioxobutanoate ethyl ester compound (1.0 mmole) in 10  
ml of methanol (MeOH) is reacted with 5 equivalents of  
sodium hydroxide (NaOH) at ambient temperature for 2  
10 hours.

The methanol is removed in vacuo. The aqueous residue is  
washed with diethyl ether (Et<sub>2</sub>O). The aqueous fraction is  
acidified by addition of 1N aqueous hydrochloric acid  
15 solution (HCl) and the milky mixture is extracted with  
two portions of ethyl acetate (EtOAc). The combined  
organic fractions are washed with brine. The organic  
layer is dried over sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), filtered and  
solvent is removed in vacuo leaving the desired  
20 dioxobutanoic acid product.

Using this or analogous methods, compounds were produced  
as set out in the following Tables, which are categorised  
according to their "R" group.

25

The Tables include IC<sub>50</sub> data and the methods for assay are  
explained after the Tables.

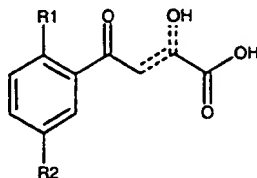
Notes to Table: NA = not active as an inhibitor at  
30 concentrations up to that stated.

ND = not done.

In the tables, where nitrogen atoms appear to be  
divalent, the presence of a hydrogen atom is implied.

**Table I**

HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids

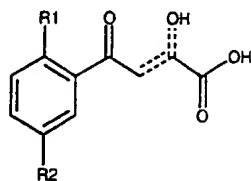


Ex. No.	R1	R2	IC 50 (μM)
1	$X_1-H$	$X_2-H$	5.6
2	$X_1-CH_3$	$X_2-H$	3
3	$X_1-H$		27.9
4	$X_1-H$		8
5	$X_1-H$		17
6		$X_2-H$	18
7	$X_1-H$		2.92
8		$X_2-H$	44



**Table I**

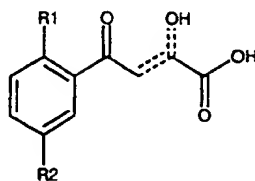
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
9		$X_2-H$	51
10	$X_1-H$		20
11	$X_1-H$		7.08
12	$X_1-H$		16.7
13	$X_1-H$		2.6
14	$X_1-H$		26
15	$X_1-H$		83.5

**Table I**

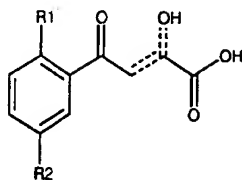
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
16		$X_2-H$	4.3
17		$X_2-H$	11.6
18	$X_1-H$		2.2
19	$X_1-H$	$X_2-CH_3$	11.9
20		$X_2-H$	0.38
21		$X_2-CH_3$	0.955

**Table I**

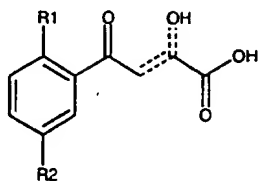
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
22	$X_1-H$		19
23	$X_1-H$	$HO-X_2$	0.94
24	$X_1-H$		19
25		$X_2-H$	28
26		$X_2-H$	26

**Table I**

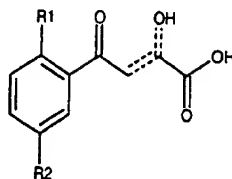
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
27		$X_2-H$	2.84
28	$X_1-H$		6.2
29		$X_2-H$	3.9
30	$X_1-H$		15
31		$X_2-H$	18

**Table I**

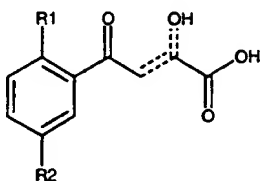
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
32			6.1
33			18.2
34			9.6
35			6.1
36			1.6
37			18

**Table I**

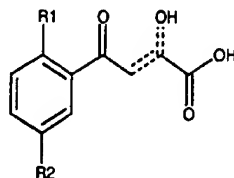
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
38		$X_2-H$	16
39		$X_2-H$	22
40		$X_2-H$	8.3
41	$X_1-H$		28.9
42		$X_2-H$	16.6
43	$X_1-H$		20
44	$X_1-H$		18.5

**Table I**

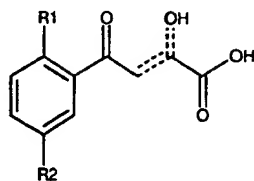
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
45	$X_1-H$		12.9
46	$X_1-H$		30.1
47	$X_1-H$		20.7
48	$X_1-H$		22
49	$X_1-H$		32
50		$X_2-H$	7.8

**Table I**

HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids

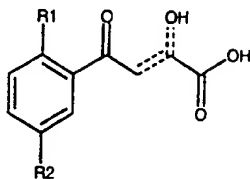


Ex. No.	R1	R2	IC 50 (μM)
51		$X_2-H$	1.9
52		$X_2-H$	10
53		$X_2-OH$	0.115
54		$X_2-Br$	2.3
55		$X_2-H$	10.8



**Table I**

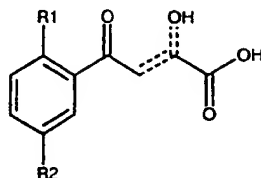
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
56			23.6
57			2.1
58			13.6
59		$X_2-H$	25.3
60	$X_1-H$	$H_3C-O-X_2$	40

**Table I**

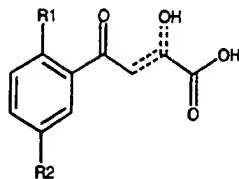
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
61	$X_1-H$		31
62	$X_1-H$	$H_2N-X_2$	10
63	$X_1-O-CH_2-CH_2-CH_2-CN$	$H_3N^+-X_2$	1.7
64	$X_1-H$		0.23
65		$X_2-H$	45
66	$X_1-H$		11

**Table I**

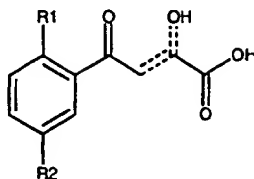
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
67	$X_1-H$		16
68	$X_1-H$		30
69		$X_2-H$	14
70	$X_1-H$		9.2

**Table I**

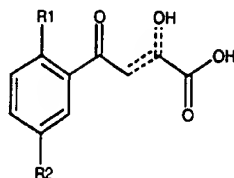
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
71	$X_1-H$		10.6
72	$X_1-H$		0.48
73	$X_1-H$		5.6
74	$X_1-H$		3.6
75	$X_1-H$		19.2
76	$X_1-H$		50

**Table I**

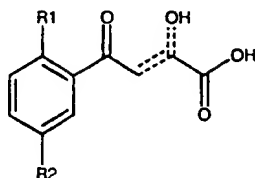
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
77	$X_1-H$		4.8
78	$X_1-H$		0.67
79	$X_1-H$		6
80	$X_1-H$		3
81	$X_1-H$		1.4

**Table I**

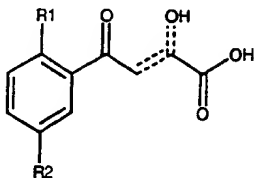
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
82	X <sub>1</sub> -H		19
83	X <sub>1</sub> -H		9.4
84	X <sub>1</sub> -H		0.95
85	X <sub>1</sub> -H		13
86	X <sub>1</sub> -H		2.05

**Table I**

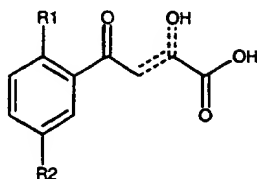
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
87	$X_1-H$	<p><chem>X2COc1cc(Cl)cc(F)c1</chem></p>	2.3
88	$X_1-H$	<p><chem>X2NS(=O)(=O)c1cc(Cl)cc(Cl)c1</chem></p>	0.7
89	$X_1-H$	<p><chem>X2COc1ccccc1Br</chem></p>	3.3
90	$X_1-H$	<p><chem>X2COc1ccccc1C(=O)O</chem></p>	1.8
91	$X_1-H$	<p><chem>X2COc1ccccc1C</chem></p>	6.2

**Table I**

HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids

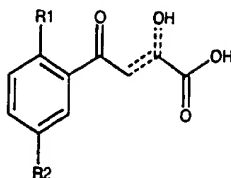


Ex. No.	R1	R2	IC 50 (μM)
92	$X_1-H$		1
93	$X_1-H$		1.9
94	$X_1-H$		5.8
95	$X_1-H$		0.48



**Table I**

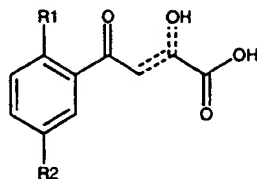
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
96	$X_1-H$	 <chem>X2OCc1ccc(cc1)-c2ccccc2</chem>	50
97	$X_1-H$	 <chem>X2OCc1ccccc1-c2ccccc2</chem>	2.8
98	$X_1-H$	 <chem>X2OCc1cc(Cl)ccc1C(=O)O</chem>	1
99	$X_1-H$	 <chem>X2OCc1cc(F)ccc1C#N</chem>	0.6

**Table I**

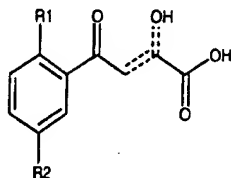
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
100	$X_1-H$		7.8
101	$X_1-H$		7
102	$X_1-H$		1.5
103	$X_1-H$		6
104	$X_1-H$		50

**Table I**

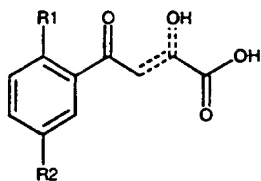
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketetoacids



Ex. No.	R1	R2	IC 50 (μM)
105	$X_1-H$		13.7
106	$X_1-H$		6.8
107	$X_1-H$		0.14
108	$X_1-H$		6.9
109	$X_1-H$		0.17

**Table I**

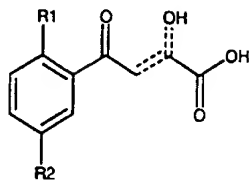
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
110	$X_1 - H$		30
111	$X_1 - H$		0.12
112	$X_1 - H$		1.33
113	$X_1 - H$		0.1
114	$X_1 - H$		0.5

**Table I**

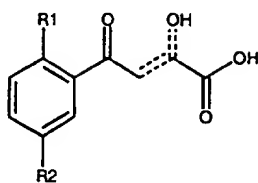
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
115	X <sub>1</sub> -H		3.7
116	X <sub>1</sub> -H		0.3
117	X <sub>1</sub> -H		0.14
118	X <sub>1</sub> -H		0.2
119	X <sub>1</sub> -H		0.049

**Table I**

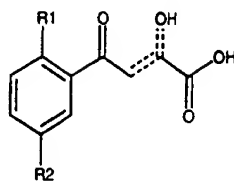
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
120	$X_1-H$		0.36
121	$X_1-H$		4
122	$X_1-H$		2
123	$X_1-H$		0.29

**Table I**

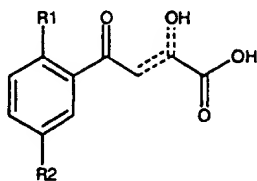
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
124	$X_1-H$		28
125	$X_1-H$		0.17
126	$X_1-H$		0.056
127	$X_1-H$		0.3

**Table I**

HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids

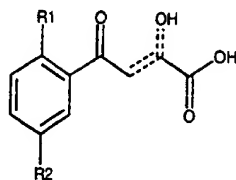


Ex. No.	R1	R2	IC 50 (μM)
128	$X_1 - H$		24
129	$X_1 - H$		1.6
130	$X_1 - H$		0.14
131	$X_1 - H$		0.78
132	$X_1 - H$		0.67



**Table I**

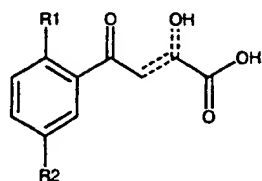
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
133	$X_1-H$		3.2
134	$X_1-H$		23
135	$X_1-H$		21
136	$X_1-H$		0.2

**Table I**

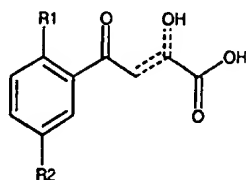
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
137	$X_1-H$		0.9
138	$X_1-H$		1.1
139	$X_1-H$		1.4
140	$X_1-H$		1
141	$X_1-H$		0.56

**Table I**

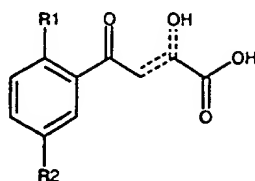
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
142	$X_1-H$		0.4
143	$X_1-H$		0.45
144	$X_1-H$		14
145	$X_1-H$		1.2
146	$X_1-H$		15

**Table I**

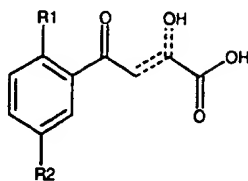
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
147	$X_1-H$		1.3
148	$X_1-H$		0.26
149	$X_1-H$		0.55
150	$X_1-H$		2.3

**Table I**

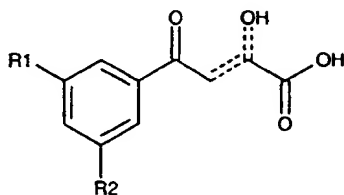
HCV-polymerase inhibitors: examples of 2,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
151	$X_1-H$		0.5
152	$X_1-F$		20
153	$X_1-H$		19
154	$X_1-H$		30

**Table II**

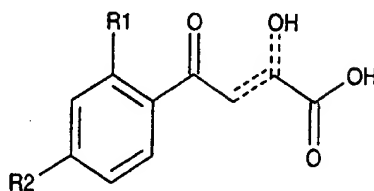
HCV-polymerase inhibitors: examples of 3,5-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
155			1.4
156			1.3
157			0.9
158			0.2
159			20
160			0.1

**TABLE III**

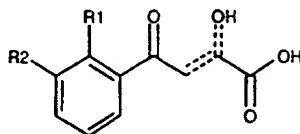
HCV-polymerase inhibitors: examples of 2,4-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
161	$\text{H}-\text{X}_1$	$\text{H}_3\text{C}-\text{X}_2$	2.8
162	$\text{H}-\text{X}_1$	$\text{HO}-\text{X}_2$	5.5
163	$\text{H}-\text{X}_1$	$\text{F}-\text{X}_2$	26
164	$\text{H}-\text{X}_1$	$\text{H}_3\text{C}-\text{CH}_2-\text{X}_2$	47
165	$\begin{array}{c} \text{CH}_3 \\   \\ \text{X}_1 \end{array}$	$\text{H}_3\text{C}-\text{X}_2$	2
166	$\text{H}-\text{X}_1$	$\text{Cl}-\text{X}_2$	20
167	$\begin{array}{c} \text{N} \\     \\ \text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{X}_1 \end{array}$	$\text{H}_3\text{C}-\text{X}_2$	0.6

**Table IV**

HCV-polymerase inhibitors: examples of 2,3-substituted phenyldiketoacids

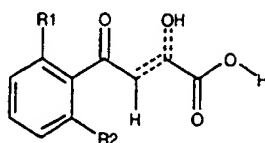


Ex. No.	R1	R2	IC 50 (μM)
168			18
169			>50
170			>50



**Table V**

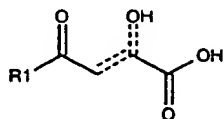
HCV-polymerase inhibitors: examples of 2,6-substituted phenyldiketoacids



Ex. No.	R1	R2	IC 50 (μM)
171			12
172			>50

**Table VIa**

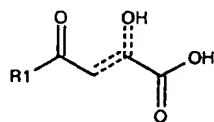
HCV-polymerase inhibitors: examples of pyrrole-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
173		21
174		13.4
175		25
176		29
177		25

**Table VIa**

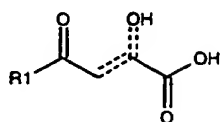
HCV-polymerase inhibitors: examples of pyrrole-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
178		17.9
179		12.8
180		93
181		30
182		30

**Table VIa**

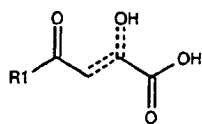
HCV-polymerase inhibitors: examples of pyrrole-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
183		32
184		6.7
185		6.3
186		24

**Table VIa**

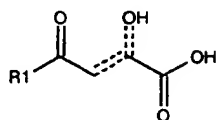
HCV-polymerase inhibitors: examples of pyrrole-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
187		36
188		12.7
189		28
190		18

**Table VIb**

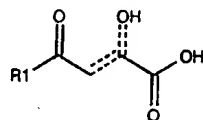
HCV-polymerase inhibitors: examples of thiophene-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
191		10
192		8.2
193		12
194		16
195		11.1
196		15
197		11
198		7.9

**Table VIb**

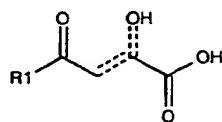
HCV-polymerase inhibitors: examples of thiophene-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
199		17
200		8.2
201		20
202		68
203		19.8
204		11
205		74
206		65

**Table VIb**

HCV-polymerase inhibitors: examples of thiophene-2-substituted diketoacids

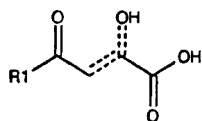


Ex. No.	R1	IC 50 (μM)
207		9.9
208		11.6
209		12.6
210		27
211		82



**Table VIb**

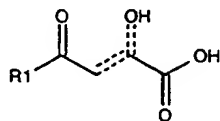
HCV-polymerase inhibitors: examples of thiophene-2-substituted diketoacids



Ex. No.	R1	IC 50 (μM)
212	<p>Chemical structure of R1 for example 212: A 4-chlorobenzyl group attached to the 2-position of a thiophene ring. The thiophene ring has a substituent X<sub>1</sub> at the 3-position.</p>	7.5
213	<p>Chemical structure of R1 for example 213: A 4-fluorophenylthio group attached to the 2-position of a thiophene ring. The thiophene ring has a substituent X<sub>1</sub> at the 3-position.</p>	5.9
214	<p>Chemical structure of R1 for example 214: A 1-phenyl-2-thienyl group attached to the 2-position of a thiophene ring. The thiophene ring has a substituent X<sub>1</sub> at the 3-position.</p>	17
215	<p>Chemical structure of R1 for example 215: A 1-(1,2,3,4-tetrahydronaphthalen-1-yl)-2-thienyl group attached to the 2-position of a thiophene ring. The thiophene ring has a substituent X<sub>1</sub> at the 3-position.</p>	15.3

**Table VIc**

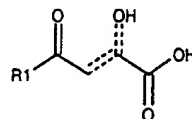
HCV-polymerase inhibitors: examples of furan-2-substituted diketoacids



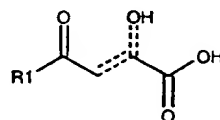
Ex.	R1	IC 50 (μM)
216		50
217		58
218		41.2

**Table VIIa**

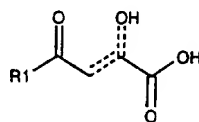
HCV-polymerase inhibitors: examples of pyrrole-3-substituted diketoacids



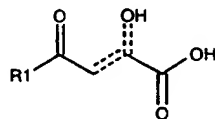
Ex.No.	R1	IC 50 (μM)
219	 <chem>Cc1cc(C)c(X1)n1</chem>	23.7
220	 <chem>N#Cc1ccc(cc1)Cn2cc(X1)cn2</chem>	4.6
221	 <chem>Cn1cc(X1)cn1Cc2ccc(F)cc2</chem>	20.6

**Table VIIb**HCV-polymerase inhibitors: examples of  
thiophene-3-substituted diketoacids

Ex.No.	R1	IC 50 (μM)
222		4
223		27
224		50
225		167
226		17
227		15
228		17.8

**Table VIIb**HCV-polymerase inhibitors: examples of  
thiophene-3-substituted diketoacids

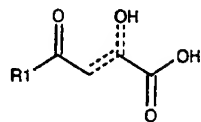
Ex.No.	R1	IC 50 (μM)
229		80
230		8.6
231		9.4
232		11.8
233		9.2
234		14.5

**Table VIIb**HCV-polymerase inhibitors: examples of  
thiophene-3-substituted diketoacids

Ex.No.	R1	IC 50 (μM)
235	<p>Chemical structure of R1 for example 235: A thiophene ring with a substituent X<sub>1</sub> at the 3-position, connected via a sulfur atom to a benzene ring with a chlorine atom at the para position.</p>	7.5
236	<p>Chemical structure of R1 for example 236: A benzothiophene system with a substituent X<sub>1</sub> at the 3-position.</p>	26

**Table VIIC**

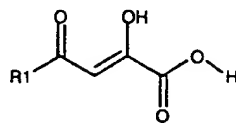
HCV-polymerase inhibitors: examples of furan-3-substituted diketoacids



Ex.No.	R1	IC 50 ( $\mu$ M)
237	<p>Chemical structure of R1 for example 237: A furan ring with a methyl group (CH<sub>3</sub>) at position 2, a substituent X<sub>1</sub> at position 3, and a methyl group (H<sub>3</sub>C) at position 4.</p>	14
238	<p>Chemical structure of R1 for example 238: A furan ring with a methyl group (CH<sub>3</sub>) at position 2, a substituent X<sub>1</sub> at position 3, and a methyl group (CH<sub>3</sub>) at position 4.</p>	47.5

**Table VIII**

HCV-polymerase inhibitors: examples of alkyl-diketoacids

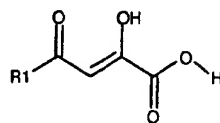


Ex. No.	R1	IC 50 (μM)
239		9.4
240		18
241		37
242		12.8
243		6.7
244		77
245		81.4



**Table VIII**

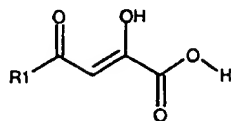
HCV-polymerase inhibitors: examples of alkyl-diketoacids



Ex. No.	R1	IC 50 ( $\mu M$ )
246	<p>Chemical structure of R1 for example 246: A p-chlorophenyl ring connected via a sulfonyl group to a piperidine ring, which is substituted with a phenyl group and an <math>X_1</math> group.</p>	18
247	<p>Chemical structure of R1 for example 247: A p-chlorophenyl ring connected via a methylene group to a piperidine ring, which is substituted with a phenyl group and an <math>X_1</math> group.</p>	45
248	<p>Chemical structure of R1 for example 248: A quinoline ring connected via a sulfonyl group to a piperidine ring, which is substituted with a phenyl group and an <math>X_1</math> group.</p>	10
249	<p>Chemical structure of R1 for example 249: A benzamide group connected via a methylene group to a piperidine ring, which is substituted with a phenyl group and an <math>X_1</math> group.</p>	60
250	<p>Chemical structure of R1 for example 250: A 3,4-dichlorophenyl ring connected via a sulfonyl group to a piperidine ring, which is substituted with a phenyl group and an <math>X_1</math> group.</p>	17
251	<p>Chemical structure of R1 for example 251: A 2,4,6-trichlorophenyl ring connected via a sulfonyl group to a piperidine ring, which is substituted with a phenyl group and an <math>X_1</math> group.</p>	21

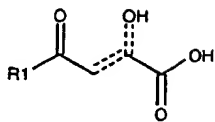
**Table VIII**

HCV-polymerase inhibitors: examples of alkyl- diketocarboxylic acids



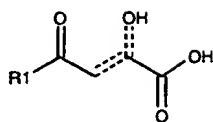
Ex. No.	R1	IC 50 (μM)
252		61
253		55
254		14
255		16.7
256		25
257		50

**Table IXa**  
most active HCV-inhibitors



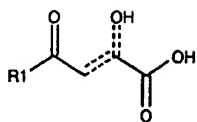
Ex. No.	R1	HCV	HIV	HBV
126		0.056	100	ND
160		0.1	NA	ND
113		0.1	90	ND
53		0.115	37	ND

**Table IXa**  
most active HCV-inhibitors



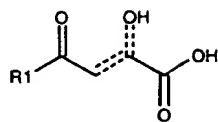
Ex. No.	R1	HCV	HIV	HBV
111		0.12	80	ND
107		0.14	58	ND
117		0.14	100	ND
109		0.17	NA	ND

**Table IXa**  
most active HCV-inhibitors



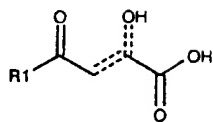
Ex. No.	R1	HCV	HIV	HBV
158		0.2	NA	ND
64		0.23	NA	ND
116		0.3	NA	ND
120		0.36	80	ND

**Table IXa**  
most active HCV-inhibitors



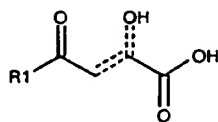
Ex. No.	R1	HCV	HIV	HBV
20		0.38	27	ND
72		0.48	NA	ND
99		0.6	50	ND
78		0.67	35	ND

**Table IXa**  
most active HCV-inhibitors



Ex. No.	R1	HCV	HIV	HBV
88		0.7	NA	ND
84		0.95	NA	ND
21		1	>50	ND
23		1	59	ND

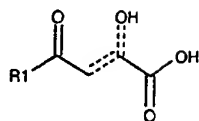
**Table IXa**  
most active HCV-inhibitors



Ex. No.	R1	HCV	HIV	HBV
112		1.33	90	ND
155		1.4	130	416
36		1.6	24	ND
90		1.8	NA	ND
165		2	NA	ND

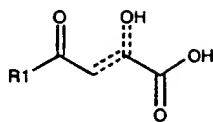


**Table IXa**  
most active HCV-inhibitors



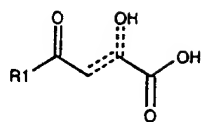
Ex. No.	R1	HCV	HIV	HBV
18		2.2	30	ND
161		2.8	320	108
80		3	NA	ND
27		3	>50	ND
7		3.3	61	6

**Table IXa**  
most active HCV-inhibitors



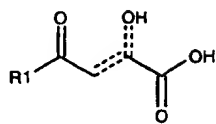
Ex. No.	R1	HCV	HIV	HBV
16		4.3	>100	ND
162		5.5	NA	ND
1		5.6	90	NA
103		6	NA	ND
243		6.7	26.8	ND

**Table IXa**  
most active HCV-inhibitors



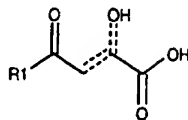
Ex. No.	R1	HCV	HIV	HBV
198	 <chem>Brc1cc(s1)C(*)</chem>	7.9	NA	ND
4	 <chem>c1ccc(cc1)COc2ccc(C*)cc2</chem>	8	>100	ND
192	 <chem>Cc1cc(C)c(s1)C(*)</chem>	8.2	NA	ND
66	 <chem>O=C(Nc1ccc(C*)cc1)c2ccccc2</chem>	11	NA	ND
19	 <chem>Cc1cccc(C*)c1</chem>	12	77	ND
179	 <chem>Oc1ccc(cc1)CN2C=CC(*)N2</chem>	12.8	NA	NA

**Table IXa**  
most active HCV-inhibitors



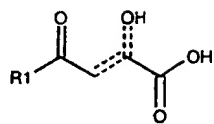
Ex. No.	R1	HCV	HIV	HBV
190	<p>The structure shows a pyrazole ring with a fluorine atom at the 3-position and an X1 substituent at the 5-position. The pyrazole ring is connected via its nitrogen atom to a methylene group, which is further connected to a para-fluorophenyl ring.</p>	18	NA	NA
24	<p>The structure shows a benzene ring with an X1 substituent at the 4-position. The benzene ring is connected via its nitrogen atom to a carbonyl group (C=O), which is further connected to a carboxylic acid group (C(=O)OH).</p>	19	71	ND
49	<p>The structure shows two benzene rings connected by a methylene group. The left benzene ring has bromine atoms at the 3 and 5 positions. The right benzene ring has an X1 substituent at the 4-position.</p>	32	NA	ND

**Table IXb**  
most active HBV-Pol-inhibitors



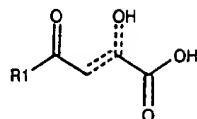
Ex. No.	R1	HCV	HIV	HBV
206		65	NA	2
205		74	NA	3.3
225		167	86	4
202		70	>100	9
196		15	50	9

**Table IXc**  
most active HIV-RT-inhibitors



Ex. No.	R1	HCV	HIV	HBV
258		>100	3.6	NA
218		41.2	11.8	40
259		>100	16	NA
40		8.3	12	NA
20		0.38	27	ND

**Table IXc**  
most active HIV-RT-inhibitors



Ex. No.	R1	HCV	HIV	HBV
8		44	19	ND

5        2.    Measurement of Inhibitory Activity

The effectiveness of the compounds set out above as polymerase inhibitors, stated above as IC<sub>50</sub> values, was assessed in screening assays as follows.

10       In initial tests, the compounds were tested to see if they were effective as inhibitors of the RNA-dependent RNA polymerase (RdRp) of hepatitis C virus (HCV). The HCV NS5B protein is the viral RdRp; compounds capable of interfering with the activity of this enzyme are thus  
15       expected to block viral replication.

Test for Inhibition of Hepatitis C Virus RdRp

WO96/37619 describes the production of recombinant HCV  
20       RdRp from insect cells infected with recombinant baculovirus encoding the enzyme. The purified enzyme was shown to possess in vitro RNA polymerase activity using RNA as template. The reference describes a  
25       polymerisation assay using poly (A) as a template and oligo(U) as a primer. Incorporation of tritiated UTP is quantified by measuring acid-insoluble radioactivity. The present inventors have employed this assay to screen the various compounds described above as inhibitors of HCV RdRp and other virally encoded polymerases.

30



5      Incorporation of radioactive UMP was measured as follows.  
The standard reaction (100  $\mu$ l) was carried out in a  
buffer containing 20mM tris/HCl pH 7.5, 5mM MgCl<sub>2</sub>, 1mM  
DTT, 50mM NaCl, 1mM EDTA, 20U Rnasin (Promega), 0.05%  
Triton X-100, 1 $\mu$ Ci[<sup>3</sup>H] UTP (40 Ci/mmol, NEN), 10 $\mu$ M UTP and  
10      10  $\mu$ g/ml poly(A). Oligo (U)<sub>12</sub> (1 $\mu$ g/ml, Genset) was added  
as a primer. The final NSSB enzyme concentration was  
20 nM. After 1 hour incubation at 22 °C the reaction was  
stopped by adding 100  $\mu$ l of 20% TCA and applying samples  
to DE81 filters. The filters were washed thoroughly with  
15      5% TCA containing 1M Na<sub>2</sub> HPO<sub>4</sub> /NaH<sub>2</sub> PO<sub>4</sub>, pH 7.0, rinsed  
with water and then ethanol, air dried, and the filter-  
bound radioactivity was measured in the scintillation  
counter. By carrying out the reaction in the presence of  
various concentrations of each of the compounds set out  
20      above it was possible to determine IC<sub>50</sub> values for each  
compound with the formula:

$$\% \text{ residual activity} = 100 / (1 + [I] / IC_{50})^s$$

where [I] is the inhibitor concentration and "s" is the  
slope of the inhibition curve.

25

#### Test for Inhibition of Hepatitis B Virus Polymerase

Analogous assays employed the polymerase of hepatitis B  
virus (HBV pol), obtained in the form of viral particles  
30      from the sera of HBV positive patients. These particles

5 contain a polymerase bound to an incomplete double stranded DNA template. In the assay the incorporation of  $^{32}\text{P}$ -dNTP is measured as radioactivity incorporated in acid insoluble precipitate.

The standard reaction (100  $\mu\text{l}$ ) was carried out in a  
10 buffer containing 50mM tris/HCl pH 7.5, 30mM MgCl<sub>2</sub>, 1mM DTT, 100 mM KCl, 0.02% Triton X-100, 1  $\mu\text{Ci}$  [ $^{32}\text{P}$ ] dCTP (300 Ci/mmol, NEN), 1  $\mu\text{M}$  dATP, dTTP, dGTP. After 1 hour incubation at 37 °C the reaction was stopped by adding 100  $\mu\text{l}$  of 20% TCA and applying samples to DE81 filters. The  
15 filters were processed and IC<sub>50</sub> values calculated as described above.

Test for Inhibition of Human Immunodeficiency Virus-1  
Reverse Transcriptase

20

Analogous assays employed the reverse transcriptase of HIV (HIV -1RT) from Boehringer Mannheim.

Incorporation of radioactive dTTP was measured as  
25 follows. The standard reaction (100  $\mu\text{l}$ ) was carried out in a buffer containing 50mM tris/HCl pH 8.2, 2.5mM MgCl<sub>2</sub>, 1mM DTT, 80 mM KCl, 5mM EGTA, 0.05% Triton X-100, 1 $\mu\text{Ci}$ [ $^3\text{H}$ ] dTTP (40 Ci/mmol, NEN), 10  $\mu\text{M}$  UTP and 10  $\mu\text{g/ml}$  poly(A)/dT (from Pharmacia). The final HIV-1RT( enzyme  
30 concentration was 1 nM. After 1 hour incubation at 37 °C

5 the reaction was stopped by adding 100  $\mu$ l of 20% TCA and  
applying samples to DE81 filters. The filters were  
processed and IC<sub>50</sub> values calculated as described above.

The results demonstrate that the compounds of the present  
10 invention are effective as inhibitors of viral  
polymerases at low micromolar concentrations.

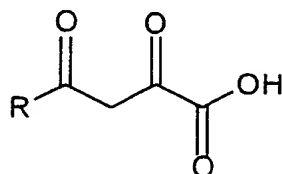
It is apparent from the tables above that a compound of  
the present invention which is effective in the  
15 inhibition of one of the RNA dependent polymerases tested  
may not necessarily be as effective in inhibiting the  
other RNA dependent polymerases. The results shown in the  
tables above indicate a general trend, although this is  
not without exception. Generally, the most active  
20 inhibitors of HCV RdRp contained a phenyl ring attached  
to the diketoacid, whereas the HIV-RT inhibitors  
contained a furanyl group and those of HBV polymerase a  
thiophene group.

25 While not wishing to be bound by any particular theory,  
the present inventors hypothesize that the diketoacid  
fragment of the compounds of the present invention  
inhibits RNA dependent polymerase activity by providing  
30 an "active site anchor" and interacting with divalent

- 5 metal cations ( $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ) required for polymerase activity. The ring system found on the left hand side of the molecule can apparently be modified in order to build specificity towards a given polymerase.

5      CLAIMS

1.    The use of a compound of formula A, or of a  
pharmaceutically acceptable salt or ester thereof,  
wherein the group R is an organic moiety containing  
10    2 to 24 carbon atoms which includes an optionally  
substituted cyclic or heterocyclic group, and  
wherein one of the atoms in the ring of the cyclic  
or heterocyclic group is directly bonded to the  
adjacent carbonyl in the diketoacid, in the  
15    manufacture of a medicament for treatment or  
prophylaxis of a viral illness in a human or animal  
by inhibition of a viral polymerase.



20

FORMULA A

2.    The use according to claim 1 wherein the medicament  
is for the inhibition of a DNA polymerase or RNA  
polymerase.

25

- 5        3.    The use according to claim 1 or claim 2 wherein the  
         medicament is for treatment or prevention of  
         infection by an RNA virus, such as a positive  
         single-stranded virus, negative single stranded  
         virus or retrovirus, or a DNA virus.
- 10       4.    The use according to claim 3 wherein the virus is  
         selected from polio virus, hepatitis C virus,  
         encephalomyocarditis, orthomyxoviruses,  
         paramyxoviruses, HIV, and hepatitis B.
- 15       5.    The use according to claim 3 wherein the medicament  
         is for the inhibition of hepatitis C virus RNA  
         dependent RNA polymerase (HCV RdRp), hepatitis B  
         virus polymerase (HBV pol), or reverse transcriptase  
20       of human immunodeficiency virus (HIV RT).
6.    The use according to any one of the following claims  
         wherein the group R is selected from:
- 25       (i)       optionally substituted aromatic groups;  
         (ii)       optionally substituted heteroaryl groups;  
         (iii)       optionally substituted cycloalkyl groups;  
         (iv)       optionally substituted cycloalkenyl  
         groups; and
- 30       (v)       optionally substituted cyclic heteroalkyl

5

groups.

10

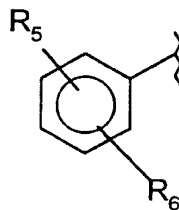
7. A compound of formula A, as set out in claim 1, or a pharmaceutically acceptable salt or ester thereof, for pharmaceutical use, wherein the group R is selected from:

15

- (i) optionally substituted aromatic groups;
- (ii) optionally substituted heteroaryl groups;
- (iii) optionally substituted cycloalkyl groups;
- (iv) optionally substituted cycloalkenyl groups; and
- (v) optionally substituted cyclic heteroalkyl groups, other than those containing a single nitrogen in the ring.

20

8. A compound, ester or salt according to claim 7 wherein the group R is an optionally substituted phenyl group of formula:



5

wherein  $R_5$  and  $R_6$  independently are selected from hydrogen and the following substituent groups:

- (a) -OH;
- (b) -SH;
- 10 (c) - halogen, such as fluorine, chlorine or bromine,
- (d) -  $\text{CO}_2\text{H}$ ;
- (e) - CN;
- (f) -  $\text{NO}_2$  ;
- 15 (g) -  $\text{NR}_1\text{R}_2$  wherein each of  $R_1$  and  $R_2$  is selected from H and lower alkyl groups having 1 to 6 carbon atoms; or  $R_1$  and  $R_2$  together form a ring including 4 to 6 carbon atoms;
- (h) -  $\text{SO}_2\text{NR}_1\text{R}_2$  where  $R_1$  and  $R_2$  are as defined
- 20 above;
- (i) - $\text{CONR}_1\text{R}_2$ , -  $\text{NR}_1\text{CO}_2\text{H}$ , or - $\text{NR}_1\text{COCOOH}$  where  $R_1$  and  $R_2$  are as defined above;
- (j) an alkyl (or alkenyl or alkynyl group) group
- 25 having 1 to 12 (2 to 12) carbon atoms, preferably 1 to 7 (2 to 7) carbon atoms optionally substituted by any one or more of the groups (a) - (i) above and/or optionally interrupted by a group selected from -O-, -S-, - $\text{NR}_3$  -,

30



5

$\begin{array}{c} \text{O} \\ || \\ -\text{C}- \end{array}$ ,  $-\text{CO}_2-$ ,  $-\text{OCO}-$ ,  $-\text{CONR}_3-$ ,  $-\text{NR}_3\text{CONR}_3-$ ,  $-\text{SO}_2$   
 $-, -\text{NR}_3\text{SO}_2-$ , and  $-\text{SO}_2\text{NR}_3-$ ; where each  $\text{R}_3$   
independently is H or lower alkyl of 1 to 6 carbon  
atoms;

10

(k) an aryl or heteroaryl group having 2 to 10  
carbon atoms optionally substituted with any  
one or more of groups (a) to (j) above;

15

(l) an aralkyl or heteroaralkyl group having 3 to  
16 carbon atoms optionally substituted with any  
one or more of groups (a) - (j) above and/or in  
which the alkyl part of the group is optionally  
interrupted by a group selected from

20

$\begin{array}{c} \text{O} \\ || \\ -\text{C}- \end{array}$  -  $\text{CO}_2-$ ,  $-\text{OCO}-$ ,  $-\text{CONR}_3-$   
 $-, -\text{NR}_3\text{CONR}_3-$ ,  $-\text{SO}_2-$ ,  $-\text{NR}_3\text{SO}_2-$ , and  $-\text{SO}_2\text{NR}_3-$ ;  
where  $\text{R}_3$  is as defined above;

25

$\begin{array}{c} \text{O} \\ || \\ -\text{C}- \end{array}$   $\text{R}_4$  where  $\text{R}_4$  is an alkyl, alkenyl, alkynyl,  
aryl, heteroaryl, aralkyl, or heteroaralkyl  
group as such groups are defined above at (j),  
(k) and (l);

30

5

(n)  $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{O}-\text{R}_4 \end{array}$  or  $\begin{array}{c} \text{O} \\ \parallel \\ -\text{O}-\text{C}-\text{R}_4 \end{array}$  where  $\text{R}_4$  is as defined above;

(o)  $-\text{OR}_4$  where  $\text{R}_4$  is as defined above;

10

(p)  $\begin{array}{c} \text{O} \\ \parallel \\ -\text{CNHR}_4 \end{array}$ ,  $\begin{array}{c} \text{O} \\ \parallel \\ -\text{NH}-\text{C}-\text{R}_4 \end{array}$  or  $\begin{array}{c} \text{O} \\ \parallel \\ -\text{NH}-\text{C}-\text{NHR}_4 \end{array}$  where  $\text{R}_4$  is as defined above;

(q)  $-\text{SO}_2\text{R}_4$  where  $\text{R}_4$  is as defined above;

15

(r)  $-\text{NHR}_4$  or  $-\text{N}(\text{R}_4)_2$  where  $\text{R}_4$  is as defined above;

(s)  $-\text{NHSO}_2\text{R}_4$  or  $-\text{SO}_2\text{NHR}_4$ , where  $\text{R}_4$  is as defined above; and

20

(t)  $-\text{SR}_4$

and each of optional substituents (j) to (t) above may optionally itself be substituted by one or more groups selected from (j) to (t).

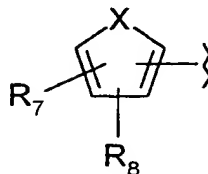
25

9. A compound, ester or salt according to claim 8 wherein the substituents  $\text{R}_5$  and  $\text{R}_6$  are independently selected from H-, -OH,  $-\text{OR}_4$ ,  $-\text{NHSO}_2\text{R}_4$ , lower alkyl, aralkyl, amino, amide, urethane or urea groups.

30

- 5        10. A compound, salt or ester according to claim 8  
         wherein the substituents  $R_5$  and  $R_6$  are independently  
         selected from H-, -OH, -OR<sub>4</sub>, and -NHSO<sub>2</sub>R<sub>4</sub>.
11. A compound, salt or ester according to claim 9 or  
10        claim 10 containing only one substituent either of  
         formula -OR<sub>4</sub> or -NHSO<sub>2</sub>R<sub>4</sub>.
12. A compound, salt or ester of any one of claims 9 to  
         11 containing a group of formula -OR<sub>4</sub> and/or -NHSO<sub>2</sub>R<sub>4</sub>  
15        selected from:  
         -OCH<sub>2</sub>Ar;  
         -O(CH<sub>2</sub>)<sub>2</sub>Ar;  
         -O(CH<sub>2</sub>)<sub>3</sub>CN;  
         -O(CH<sub>2</sub>)<sub>3</sub>C≡CH; and  
20        -NHSO<sub>2</sub>Ar;  
         wherein Ar is an optionally substituted aryl or  
         heteroaryl group.
13. A compound, salt or ester, according to any one of  
25        claims 8 to 12 having a single substituent at a  
         position ortho- or meta- to the diketoacid group.
14. A compound, salt or ester according to any one of  
         claims 8 to 12 having two substituents at the 2,5-;  
30        3,5-; or 2,4-positions.

- 5        15. A compound, salt or ester according to claim 7  
         wherein the group of formula R has the formula:



         and each of R<sub>7</sub> and R<sub>8</sub> is independently selected from  
         hydrogen or from the list of substituent groups set  
10        out at claim 8, and X is O, S, NH or NR<sub>4</sub>, where R<sub>4</sub> is  
         as defined above.

16. A compound, salt or ester according to claim 15  
         which is a pyrrole-2-substituted diketoacid, a  
15        pyrrole-3-substituted diketoacid, a thiophene-2-  
         substituted diketoacid, or a thiophene-3-substituted  
         diketoacid.

17. A compound, salt or ester according to claim 16  
20        which is a pyrrole substituted diketoacid in which  
         each of R<sub>7</sub> and R<sub>8</sub> is hydrogen.

18. A compound, salt or ester according to any one of  
         claims 15 to 17 which is a pyrrole substituted  
25        diketoacid having X=NR<sub>4</sub> and wherein R<sub>4</sub> is selected

- 5           from optionally substituted or interrupted, alkyl  
          aryl or aralkyl groups.
19.   A compound, salt or ester according to claim 7  
      wherein R is selected from cyclopropyl-,  
10       cyclopentyl-, cyclohexyl-, cyclopentenyl-,  
      cyclohexenyl and adamantyl groups, any of which may,  
      optionally, be substituted.
20.   A pharmaceutical composition comprising a compound,  
15       salt or ester of any one of claims 7 to 19 in  
      combination with a pharmaceutically acceptable  
      excipient, diluent or carrier.
21.   Use, according to any one of claims 1 to 6, of a  
20       compound, salt or ester according to any one of  
      claims 7 to 19.
22.   A use according to any one of claims 1 to 6 or 21  
      wherein the medicament further comprises one or more  
25       other agents for the treatment of viral infections.
23.   A method of inhibiting a viral polymerase and/or of  
      treating or preventing a viral illness by inhibiting  
      a viral polymerase, the method comprising  
30       administering to a human or animal subject suffering

5           from the condition a therapeutically or  
prophylactically effective amount of the compound of  
formula A set out in claim 1, or of a  
pharmaceutically acceptable salt or ester thereof.

10       24. A compound of formula A, as set out in claim 1 or a  
pharmaceutically acceptable salt or ester thereof  
wherein the group R is selected from:

- (i)           optionally substituted aromatic groups;
- (ii)          optionally substituted heteroaryl groups;
- 15       (iii)       optionally substituted cycloalkyl groups;
- (iv)          optionally substituted cycloalkenyl  
groups; and
- (v)          optionally substituted cyclic heteroalkyl  
groups, other than those containing a  
20       single nitrogen in the ring.

## INTERNATIONAL SEARCH REPORT

International Application No

PC1/GB 99/02446

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C59/76 C07D207/30 C07D307/34 C07D333/04 A61K31/19  
 A61K31/335 A61K31/40 A61K31/38

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 475 109 A (SELNICK HAROLD G ET AL) 12 December 1995 (1995-12-12) cited in the application table 4	1-6,24
X	--- TOMASSINI ET AL.: "Inhibition of ...." ANTIMICROB. AGENTS CHEMOTHERAP., vol. 38, no. 12, 1994, pages 2827-2837, XP002119719 table 1	1-14,24
X	--- DE 32 14 082 A (ROUSSEL UCLAF) 4 November 1982 (1982-11-04) the whole document	7-14,24
X	--- US 4 337 258 A (ROONEY CLARENCE S ET AL) 29 June 1982 (1982-06-29) claims 1-4 --- -/--	7,24

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

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"Z" document member of the same patent family

Date of the actual completion of the international search

21 October 1999

Date of mailing of the international search report

10/11/1999

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# INTERNATIONAL SEARCH REPORT

International Application No  
PC1/GB 99/02446

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEILSTEIN INFORMATION SERVICE: FILE: XFIRE, XP002119720 see the compounds attached the whole document -----	24

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page 2 of 2



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Information on patent family members

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